Final Report

Study Title:

Daphnia magna, acute immobilization test

Author:
Osamu Saika

Study Completed on:
July 22, 2010

Test Facility:
Nisso Chemical Analysis Service Co., Ltd. (NCAS)
Odawara Laboratory
345 Takada, Odawara, Kanagawa 250-0216, Japan

Sponsor:

Study Number:
NCAS 10-064

GLP Compliance Statement

Study No.: NCAS 10-064

Study title: : *Daphnia magna*, acute immobilization test

This study was carried out in accordance with the following good laboratory practice regulation;

Standard for the test facility conducting tests concerning new chemical substances, etc. (Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, No. 1121003, November 21, 2003; Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry, No.3, November 17, 2003; Environmental Policy Bureau, Ministry of the Environment, No. 031121004) The latest amendment: July 4, 2008.

The final report was prepared faithfully and consistently with the raw data obtained.

Study Director: (signature) (seal) (July 22, 2010)

Osamu Saika
Nisso Chemical Analysis Service Co., Ltd.
Odawara Laboratory

The original signature page of GLP Compliance Statement follows on page 3.

The original GLP Compliance Statement

The English translation of GLP Compliance Statement appears on page 2.

GLP 適合陳述書

試験番号： NCAS 10-064

試 験 名：

この試験は「新規化学物質等に係る試験を実施する試験施設に関する基準について」平成 15 年 11 月 21 日薬食発第 1121003 号、平成 15・11・17 製局第 3 号、環保企発第 031121004 号（最終改正 平成 20 年 7 月 4 日）に従って実施した。

この試験はこの最終報告書に述べられた方法により行われ、この最終報告書は試験実施により得られた生データを正確に反映したものである。

試験責任者： 雜賀 修 印 2010 年 7 月 22 日

雜賀 修

（株）日曹分析センター 小田原事業所

Quality Assurance Statement

Study Number: NCAS10-064

Study Title: *Daphnia magna*, acute immobilization test

Quality Assurance inspections of the study referred above were conducted according to the appropriate GLP regulations and the standard operating procedures (SOPs) of the Quality Assurance Unit (QAU). The results of the inspections were reported to the study director and the facility management on the following dates.

Items inspected	Dates (Month/Day/Year)		
	Inspected	Reported to	
		Study Director	Management
Protocol	5/21/2010	5/24/2010	5/24/2010
Experimental procedures			
· Acquisition, breeding and keeping of daphnids	5/25/2010	5/27/2010	5/27/2010
· Preparation of the test solutions	5/24, 25/2010	5/27/2010	5/27/2010
· Exposure of daphnids to the test solutions	5/24, 25/2010	5/27/2010	5/27/2010
· Observation of daphnids	5/25/2010	5/27/2010	5/27/2010
· Analytical sampling and treatment	5/24, 26/2010	5/27/2010	5/27/2010
· Analysis of the concentrations	5/24, 26/2010	5/27/2010	5/27/2010
Raw Data	7/5-7/2010	7/7/2010	7/7/2010
Draft Report	7/5-7/2010	7/7/2010	7/7/2010
Final Report	7/22/2010	7/22/2010	7/22/2010

The QAU found that the study was performed according to the protocol and SOPs, the reported methods and procedures were actually used, and the results accurately reflect the recorded data.

QAU Manager: (signature) (seal) (July 22, 2010)

Ken Watabe

Nisso Chemical Analysis Service Co., Ltd.

The original signature page of Quality Assurance Statement follows on page 5.

The original Quality Assurance Statement

The English translation of Quality Assurance Statement appears on page 4.

信頼性保証書

試験番号： NCAS 10-064

試 験 名：

上記試験の信頼性保証の監査または査察を適用 GLP および信頼性保証部門（QAU）の SOP に基づいて実施した。監査または査察の結果は、以下の日付で試験責任者および運営管理者に報告した。

監査または査察項目	日付（月/日/年）		
	監査または査察日	報告日	
		試験責任者	運営管理者
試験計画書	5/21/2010	5/24/2010	5/24/2010
実験操作			
・ミジンコの入手、飼育と継代	5/25/2010	5/27/2010	5/27/2010
・試験溶液の調製	5/24, 25/2010	5/27/2010	5/27/2010
・試験溶液への暴露	5/24, 25/2010	5/27/2010	5/27/2010
・ミジンコの観察	5/25/2010	5/27/2010	5/27/2010
・分析試料の採取および処理	5/24, 26/2010	5/27/2010	5/27/2010
・濃度分析	5/24, 26/2010	5/27/2010	5/27/2010
生データ	7/5-7/2010	7/7/2010	7/7/2010
報告書草案	7/5-7/2010	7/7/2010	7/7/2010
最終報告書	7/22/2010	7/22/2010	7/22/2010

QAU は、この試験が試験計画書および SOP に従って行われ、報告された方法や手段が実際に使われたものであり、結果は記録されたデータを正確に反映していることを確認した。

QAU 責任者 渡部健 印 2010年7月22日

渡部 健

（株）日曹分析センター

Study Information

Study No.:	NCAS 10-064
Study Title:	*Daphnia magna*, acute immobilization test
Report No.:	NCAS 10-064
Sponsor:	

Test Facility:	Nisso Chemical Analysis Service Co., Ltd. Odawara Laboratory 345 Takada, Odawara, Kanagawa 250-0216, Japan TEL 81-465-42-5268, FAX 81-465-42-3586
Study Director:	Osamu Saika
Experimenter:	Satoko Ogawa (test solution preparation, exposure, observation and analysis of water parameter) Shinpei Oonuki (analysis of concentration of test substance) Chiho Nakamura (maintenance of test organisms)

Study Initiation Date:	May 21, 2010
Experimental Start Date:	May 24, 2010
Exposure Period:	May 24, 2010–May 26, 2010
Experimental Completion Date:	May 26, 2010
Study Completion Date:	July 22, 2010

Test Guideline:	Standard for the testing methods concerning new chemical substances. (Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, No.1121002, November 21, 2003; Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry, No.2, November 13, 2003; Environmental Policy Bureau, Ministry of the Environment, No. 031121002) The latest amendment: November 20, 2006, *Daphnia sp.*, Acute Immobilization Test
Archiving:	All the documents of this study will be retained in the archives of the test facility for 10 years after the completion of the study, but the place for storage after that will be decided on discussion with the sponsor. The test substance will be retained in the

test facility at least for 10 years after the completion of the study, but only as long as the quality of the substance affords evaluation.

Deviations from the SOPs and the protocol: None

Circumstance / matter that may affect the reliability of the test results: None

Table of Contents

Summary

The *Daphnia magna*, acute immobilization study (static and open system) was performed to estimate the effect of the test substance, A 24.0% aqueous solution of was used as the test substance. In this report, the concentrations of were expressed as , active ingredient, unless otherwise specially stated. The measured concentrations of the test substance in the test solutions at nominal concentrations of 6.26, 12.5, 25.0, 50.0 and 100 mg/L were 102−106 % of the nominal at the start of the exposure and 97.8–101 % of the nominal at the end of the exposure. Since the variability of concentrations was within ± 20% of the nominal, the median immobilization concentrations (EC_{50}) were calculated on the basis of the nominal concentrations as the test concentrations during the exposure period. The 24- and 48-hour EC_{50} are presented below. The 95% confidence interval was not calculated since the EC_{50} was determined by graphical method.

Exposure duration	EC_{50} (mg/L)
24 hours	89
48 hours	71

Introduction

This study was conducted in accordance with the following requirement to determine the median immobilization concentrations (EC_{50}) by exposing the *Daphnia magna* to the test solutions that contained for 48 hours.

- Standard for the testing methods concerning new chemical substances. (Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, No.1121002, November 21, 2003; Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry, No.2, November 13, 2003; Environmental Policy Bureau, Ministry of the Environment, No. 031121002) The latest amendment: November 20, 2006, *Daphnia sp.*, Acute Immobilization Test

Materials and Methods

1. Test substance

   Name:

   Abbreviated name:

   Product name:

   Chemical name:

Structure:
CAS No.:
Molecular formula:
Molecular weight:
Lot No.:
NCAS retrieval No.: STD-1150
Purity: Solid content; 24.0% aqueous solution

Impurities:).85% (as against solid content), Cl ion; 35 ppm
In this report, the concentrations of the test substance were expressed as those of active ingredient, unless otherwise specially stated. was defined as the solid contents except water in this study.

Appearance:
Obtained from:
Acquisition volume: 420 g
Acquisition date: January 18, 2010
Expiration date: January 18, 2013
Storage conditions: Stored in a refrigerator with a polypropylene container
Water solubility: Soluble
Stability information: Stable in water
Summary of risk and harmfulness: Causes skin and eye irritation. Wear protective gloves, goggles and mask when it is used (from MSDS).

2. Verification of the test substance
The identity of the test substance was verified by comparing a ^{19}F-NMR spectrum which was measured before the exposure initiation with another spectrum which was supplied by the sponsor (Figure 1 and 2). This verification was conducted in another study, NCAS 10-022 "Determination of the Concentration of in Dosing Solutions".

3. Verification of stability of test substance
The stability under storage condition was verified by confirming identity between two ^{19}F-NMR spectra which were measured before and after the exposure period. The verification of stability of the test substance was conducted in another study, NCAS 10-066
Acute toxicity Study in *Oryzias latipes*".

4. Verification of solubility of test substance
Since the test substance was a 24% aqueous solution, its solubility was not confirmed.

5. Reagents and apparatus
Dechlorinated tap water: Odawara City tap water was dechlorinated by activated charcoal treatment and aerated sufficiently with an air pump before the test. Quality of the

water was confirmed to meet the standard criteria of 3rd-class fisheries water (Report No.: NCAS 09-064NG, provided from Chiba Pharmacist Association Inspection Center Foundation, Sample No.: D9C-8079, Sampling Day: December 8, 2009). The total hardness and the concentration of the residual chlorine were measured before the test and it was confirmed that the total hardness as $CaCO_3$ was 55 mg/L, within the prescribed range (total hardness as $CaCO_3$ was 40–100 mg/L), and the concentration of the residual chlorine was <0.01 mg/L.

Ion exchange water:	Tap water was distilled with a Barnstead water distilling apparatus (WDA-15S, Isuzu Seisakusho Co., Ltd.) and then purified with an ultrapure water system (LV-08, Toray Co., Ltd.)
Acetonitrile:	HPLC grade (Wako Pure Chemical Industries, Ltd.)
Formic acid:	Special grade (Wako Pure Chemical Industries, Ltd.)
Hardness measurement kit:	WAD-TH (Kyoritsu Chemical-check Lab., Corp)
Balances:	AX205, AE240 and XP205 (Mettler Toledo)
Thermostat water bath:	BF200 (Yamato Kagaku Co., Ltd.),
Cooling device:	LTC-450 (As One Co., Ltd.)
Thermo recorder:	SK-L200T (SATO KEIRYOKI MTG Co., Ltd.)
Dissolved oxygen meter:	720A (Thermo Fisher Scientific Inc.), OM-51 (HORIBA, Ltd.)
pH meter:	720A (Thermo Fisher Scientific Inc.), D-51(HORIBA, Ltd.)
Illuminometer:	LX-1330D (Custom Co., Ltd.)
Thermometer:	Standard thermometer; measuring temperature range from 0 to 50°C, minimum scale 0.1°C, (Ando Keiki Co., Ltd.)
Residual chloride meter:	Digital residual chloride tester DCT-200, Digital residual chloride tester DCT-300 (Takumina Co., Ltd.)
Centrifuge:	KN-70 (Kubota Co.)
Micropipette:	Eppendorf Research V (Eppendorf Co., Ltd.)
Centrifuge tube:	10-mL glass test tube with ground-in stopper
LC/MS/MS:	Acquity/Quattro micro API (Waters Corp.)

6. Test organism

6.1 Test organism

Species:	*Daphnia magna*
Obtained from:	National Institute for Environmental Studies (16-2, Onogawa, Tsukuba-City, Ibaraki, on June 20, 2008)
Growth stage	Neonates (less than 24 hours old) were obtained from 17 days old parent animals
Sensitivity:	The background data of the 48-hour EC_{50} value of the reference substance (potassium dichromate, special grade, Wako Pure Chemical Industries, Ltd.) in our laboratory; 0.18–0.24 mg/L (n=4, July 2008–February 2010).

6.2 Breeding conditions of parent animals to obtain test organisms

Young individuals were removed thoroughly from the stock cultures and then the neonates born by the next day were transferred to another vessel. These neonates (released on May 7, 2010) were maintained as parent of the test organisms. After the parents started to breed, young individuals were removed at least twice a week. On the day before the exposure, young individuals were removed thoroughly from the breeding vessel, and the neonates released after that (less than 24 hours old) were used for the test (exposure initiation date: May 24, 2010). In breeding period, the parent animal mortality was 0%, and no abnormal signs such as the production of ephippia or males were shown.

Breeding water:	Dechlorinated tap water
Culture density:	10 individuals (transferred at the start of breeding) / 500 mL breeding water
Breeding container:	500-mL glass container
Water temperature:	19.8–19.9 °C
pH:	7.4–7.8
Total hardness:	55–65 mg/L (as $CaCO_3$)
Dissolved oxygen concentration:	7.3–7.7 mg/L (≥82% of saturation)
Lighting:	fluorescent light (530–630 Lux), 16 hour light / 8 hours dark
Feed:	unicellular green algae (*Chlorella vulgaris*, Raw Chlorella V12, Chlorella industry Co., Ltd)
Feeding volume:	Foods were given at a rate of 0.1–0.2 mg-C per individual per day. Before the start of the production, the animals were fed with an adequate volume of food.
Exchange of the breeding water:	at least once a week
Test room:	B6

7. Acute immobilization study

7.1 Test conditions

Exposure system:	Static
Exposure duration:	48 hours
Test vessels:	100-mL tall glass beaker
Volume of test solutions:	100 mL / vessel
Replicates:	4 vessels / group
Number of daphnids used:	20 individuals / group (5 individuals / vessel)
Dilution water:	Dechlorinated tap water (the concentration of the residual chlorine was <0.01 mg/L, the total hardness were 55 mg/L (as $CaCO_3$)).
Test temperature:	19.6–19.7°C (the temperature of the test solutions: test vessels were placed in a water bath installed a thermostat water bath) 19.9–20.0°C (water temperature in water bath during the exposure

	period)
pH:	7.8–7.9
Dissolved oxygen concentration: 7.3–9.1 mg/L (≥82% of saturation)	
Lightning:	fluorescent light (520–890 Lux), 16 hour light / 8 hours dark
Feeding:	None
Test room:	B3

7.2 Selection of the test concentrations and exposure systems.

A 48-hour range-finding test (Report No.; NCAS 10-063NG) was conducted at nominal concentration of 10 and 100 mg/L under static condition, and the percentages of immobilization were 0 and 100% respectively. The nominal concentrations were maintained for 48 hours. Therefore, the definitive test was conducted at 5 nominal concentrations (common ratio; 2.0) of 6.26, 12.5, 25.0, 50.0 and 100 mg/L. One control group using dechlorinated tap water was also made.

7.3 Preparation of test solutions

Test solutions were prepared before use on the day of exposure. An amount of test substance (417 mg as aqueous solution; 100 mg as) was weighed and transferred to a 1000-mL volumetric flask and dissolved in dechlorinated tap water (Two solutions were prepared, one was for stock solution and another was for the test solutions). These stock solutions of 31.3, 62.5, 125 and 250 mL were separately transferred to 500-mL flasks and brought to volume with dechlorinated tap water to prepare the test solutions of 6.26, 12.5, 25.0 and 50.0 mg/L respectively. After preparation, the conditions (appearance) of the test solutions were recorded. Four vessels containing 100 mL of solution were used for each exposure group. For the control group, four vessels containing 100 mL of the dechlorinated tap water were used.

7.4 Analysis of test concentrations

7.4.1 LC/MS/MS conditions

Apparatus:	Acquity / Quattro micro API (Waters)
Column:	Acquity UPLC BEH C18, 2.1 mm i.d. × 50 mm, particle diameter; 1.7 μm (Waters)
Mobile Phase:	Acetonitrile + 0.1% formic acid aqueous solution (v/v) = 50 + 50 (v/v)
Flow rate:	0.3 mL/min
Column temperature:	40°C
Injection volume:	5 μL
Ionization mode:	ESI, Negative
Monitoring ion:	180.33 > 96.90 (quantification), 180.33 > 77.84 (confirmation)

7.4.2 Preparation of the standard solutions and the calibration curves

An amount (5.05 mg) of the test substance (as) was correctly weighed and transferred into a 50-mL volumetric flask and dissolved in 50% acetonitrile aqueous solution to prepare a 101 mg/L standard stock solution. This standard stock solution was then further diluted with the same solvent

to prepare 1.01, 2.02, 3.03, 4.04 and 5.05 mg/L standard solutions. These standard solutions were analyzed by using the LC/MC/MC conditions described in section 7.4.1. Calibration curves were prepared by plotting the peak areas *vs.* the concentrations of in the standard solutions and the linear regression equations and correlation coefficients (r) were calculated by using a computer program of LC/MS/MS (Mass Lynx v 4.1). The accuracy (%) for each calibration solution was calculated by inverse regression method. The weighting of 1/x was carried out. The standard solutions used to construct calibration curves were prepared before use.

7.4.3 Analysis of the concentration of the test substance in the test solutions

The test solutions were analyzed at the start and end of the exposure. Samples were mixed with acetonitrile at the rate of 1:1(v/v) and centrifuged (3000 rpm, 10 min). The supernatant fluids obtained were diluted with 50% acetonitrile aqueous solution to be appropriate concentration which was approximated in the middle of the range of the calibration curve, and then analyzed by using the LC/MS/MS conditions as described in section 7.4.1.

Since variations of the measured concentrations of the test substance at start and end of the exposure were within ±20% of the nominal, the nominal concentrations were regarded as the test concentrations during the exposure period.

The samples from the control group were also mixed with acetonitrile at the rate of 1:1(v/v) and centrifuged (3000 rpm, 10 min) and then analyzed by the same procedures to verify that any interfering peak did not appear at the retention time of the test substance on the chromatogram.

7.5 Validation study of the analysis of the test substance concentration in the test solutions

7.5.1 Validation of the calibration curve

The accuracy was calculated by finding the regression equation and correlation coefficient (r) of calibration curve, and then determining the quantitative value using inverse estimation of regression. The criteria for the calibration curve were described below.

1. The correlation coefficient is more than 0.990.
2. The accuracy for the lowest concentration solution is within ±20%, and that for other solutions is within ±15%.

7.5.2 Validation of the repeatability

Analytical method was validated at the exposure initiation by a repeatability test without any recovery tests, because extract operation was not conducted. The concentrations of the test substance in the highest and lowest concentration were calculated by the same procedure as described in section 7.4.3 (n=3). The analytical method was validated by the coefficient of variations of the measured test concentrations (n=3) which was within ±10%.

7.6 Exposure procedure

The test was initiated when daphnids were assigned to the test vessels that contained the test solutions.

The conditions (appearance) of the test solutions were recorded at the start, and at 24 and 48 hours

after the beginning of the exposure. Water temperature, dissolved oxygen and pH were measured at the start and end of the exposure. At the exposure initiation, one vessel which was prepared in addition to 4 replicate test solutions was measured. Temperature in the thermostat water bath, in which the test vessels ware placed, was continuously recorded during the exposure period with the temperature recorder. At the exposure termination, the measurement was conducted in one vessel in each exposure and control group.

Daphnids were observed to check if there were immobilized daphnids, any abnormal behavior or appearance at 24 and 48 hours after the beginning of the exposure. Daphnids were considered to be immobilized when the daphnids did not swim within 15 seconds after gentle agitation of the test vessel. If daphnids were touched on the bottom of the vessel or trapped on water surface, it was indicated immobilization.

7.7 Calculation of the median immobilization concentration (EC_{50})

The EC_{50} were calculated from the number of immobilization and the number of daphnids used by graphical method. The values of EC_{50} and 95% confidence limit were shown as 2 significant figures.

Results

1. Validation test of the test concentration analysis

1.1 Validation of the linearity of the calibration curve

A typical calibration curve is shown in Figure 4 and a typical HPLC chromatogram of the standard solution (5.05 mg/L, at the start of the exposure) is shown in Figure 5 (measured in the study NCAS 10-066). The coefficients of correlation (r) were more than 0.990 both at the start and end of the exposure. The all variations of the accuracy of the lowest standard samples were within ±20% and those of other standard samples were within ±15%. Based on the above results, the linearity of the calibration curves were determined as acceptable.

1.2 Validation of the repeatability

The results of the repeatability test are shown in Table 1. A typical chromatogram of the repeatability test (nominal concentration: 100 mg/L) is shown in Figure 6. Chromatogram of the control group is shown in Figure 7.

The Coefficients of variation (n=3) of the test solutions were all within 10%. On the chromatogram of the control group, no interfering peak appeared at the retention time of the test substance. On the basis of these results, the analytical method of the test substance concentration in the test solution was determined to be valid in this study.

2. The concentrations of the test substance in the test solutions

The analytical results of the test substance concentrations in the test solutions are presented in Table 2

and 9 respectively.

The measured concentrations of the test substance in the test solutions were 102–106% of the nominal at the exposure initiation and 97.8–101% of the nominal at the exposure termination. The test substance concentrations in the test vessels were stable during the exposure period. Since the variability of the measured concentrations of the test substance was within ±20% of the nominal, the nominal concentrations were used as the test concentrations.

3. Immobility

The number and percentage of immobilized daphnids in each test group are shown in Table 3, and the relationship between the concentrations of the test substance and percentage of immobilization are shown in Figure 10.

No immobility was observed in the 6.26, 12.5, 25.0 and 50.0 mg/L groups both after 24 and 48 hours of the exposure. Immobility in the 100 mg/L group after 24 hours of the exposure was 60% and after 48 hours was 100%. In addition, some abnormal behavior was observed among daphnids in the 50.0 and 100 mg/L group after 24 hours and in the 25.0 and 50.0 mg/L group after 48 hours of the exposure.

In the control group, no abnormality was observed during the exposure period.

4. EC_{50} and 95% confidence interval

The values of EC_{50} are presented in Table 4.

The 24-hour and 48-hour EC_{50} value were determined to be 89 mg/L and 71 mg/L, respectively, by using graphical method. The 95% confidence interval was not calculated.

5. Observations of the test solutions

The observations of the test solutions were shown in Table 5.

All solutions in exposure and control groups were colorless and transparent during the exposure period.

6. Temperature, pH and dissolved oxygen concentration in the test solutions

The measurements of temperature, pH and dissolved oxygen concentration in the test solutions are presented in Table 6.

Throughout the exposure period, the temperatures in the test solutions ranged from 19.6 to 19.7°C and the pH ranged from 7.8 to 7.9. The dissolved oxygen concentrations ranged from 8.8 to 9.1 mg/L (≥99% of saturation) at the exposure initiation and from 7.3 to 7.7 mg/L (≥82% of saturation) at the exposure termination, therefore maintained within the prescribed range of ≥3.0 mg/L.

7. Validity of the test

The validity of the test was confirmed because there were no items met the following situation;

- More than 10% of daphnids in the control group was immobilized.
- Dissolved oxygen concentrations at the end of the exposure were less than 3 mg/L.

Conclusions

The acute immobilization study (static and open system) was performed by exposing *Daphnia magna* to

It is concluded that the 48-hour EC_{50} values is 71 mg/L.

Tables and Figures

Table 1 Result of validation of the analysis

Nominal concentration (mg/L)	Measured concentration (mg/L)	Mean measured concentration (mg/L)	Coefficient of variation (%)
100	106 104 107	106	1.44
6.26	6.34 6.46 6.66	6.49	2.49

Table 2 Concentration of test substance in test solution

Nominal concentration (mg/L)	Measured concentration (mg/L)	
	0 hour	72 hours
Control	N.D.	N.D.
6.26	6.48 (104)	6.12 (97.8)
12.5	12.7 (102)	12.3 (98.4)
25.0	25.5 (102)	25.3 (101)
50.0	53.0 (106)	49.3 (98.6)
100	105 (105)	98.0 (98.0)

( ): Percent of nominal concentration (%)

N.D.: Not detected

Table 3 Number of immobilization and percentage of immobilization

n=20

Nominal concentration (mg/L)	Number of immobilization (Percentage of the immobilization (%))	
	24 hours	48 hours
Control	0 (0)	0 (0)
6.26	0 (0)	0 (0)
12.5	0 (0)	0 (0)
25.0	0 (0)	0 (0)
50.0	0 (0)	0 (0)
100	12 (60)	20 (100)

Table 4 EC_{50} and 95% confidence interval

Exposure duration	EC_{50} (mg/L)	95% confidence interval (mg/L)
24 hours	89	—*
48 hours	71	—*

*: Since the EC_{50} were determined by graphical method, the 95% confidence interval was not calculated.

Table 5 Observations of the test solutions

Nominal concentration (mg/L)	0 hour	24 hours	48 hours
Control	Colorless and transparent	Colorless and transparent	Colorless and transparent
6.26	Colorless and transparent	Colorless and transparent	Colorless and transparent
12.5	Colorless and transparent	Colorless and transparent	Colorless and transparent
25.0	Colorless and transparent	Colorless and transparent	Colorless and transparent
50.0	Colorless and transparent	Colorless and transparent	Colorless and transparent
100	Colorless and transparent	Colorless and transparent	Colorless and transparent

Table 6 Temperature, pH and dissolved oxygen concentration in the test solutions

Nominal concentration (mg/L)	Temperature (°C)		pH		Dissolved oxygen concentration* (mg/L)	
	0 hour	48 hours	0 hour	48 hours	0 hour	48 hours
Control	19.7	19.7	7.8	7.8	8.9 (100)	7.4 (83)
6.26	19.7	19.7	7.9	7.9	8.8 (99)	7.4 (83)
12.5	19.6	19.7	7.9	7.8	9.0 (101)	7.3 (82)
25.0	19.7	19.7	7.8	7.8	9.0 (101)	7.6 (85)
50.0	19.7	19.7	7.9	7.9	9.0 (101)	7.7 (87)
100	19.7	19.7	7.9	7.9	9.1 (102)	7.6 (85)

*: Figures in parentheses indicate the percentages of saturation.

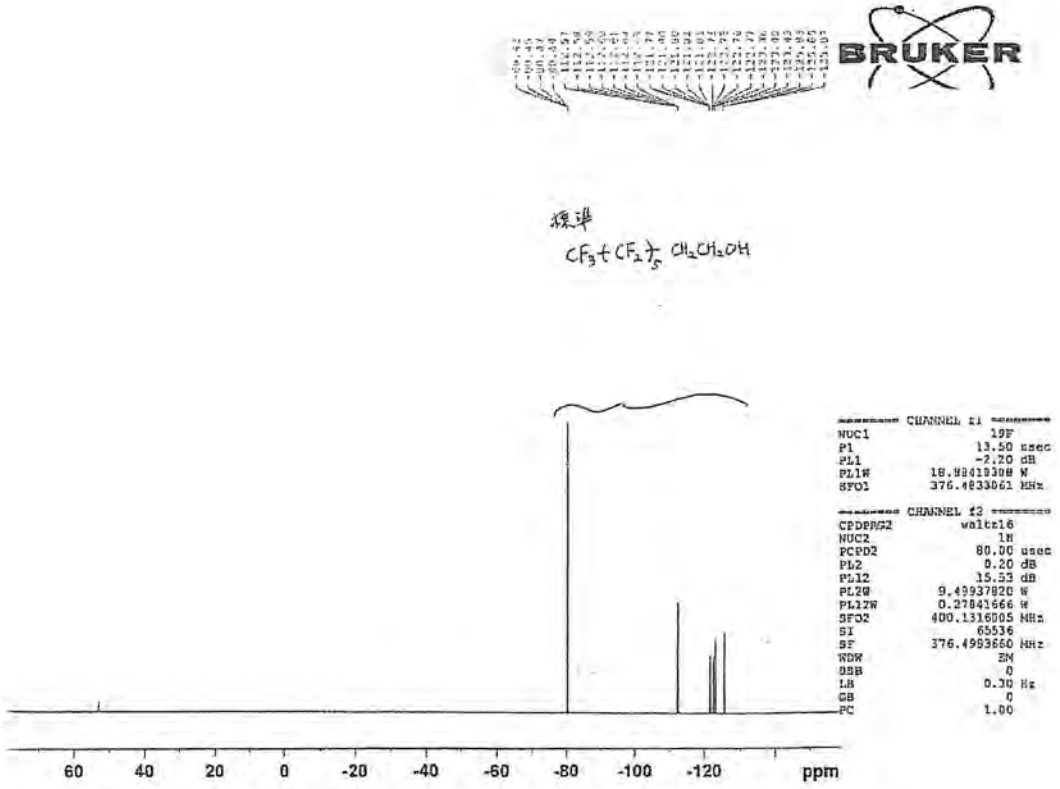

Figure 1 NMR spectrum of supplied by Sponsor

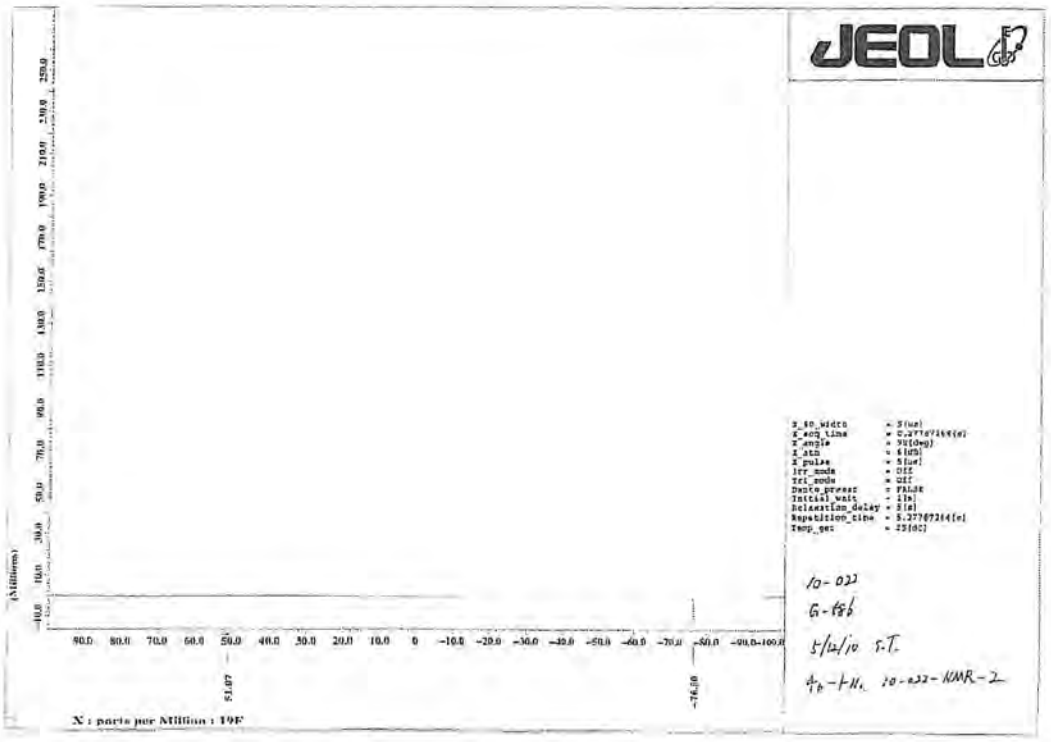

Figure 2 NMR spectrum of the test substance (before the exposure initiation)

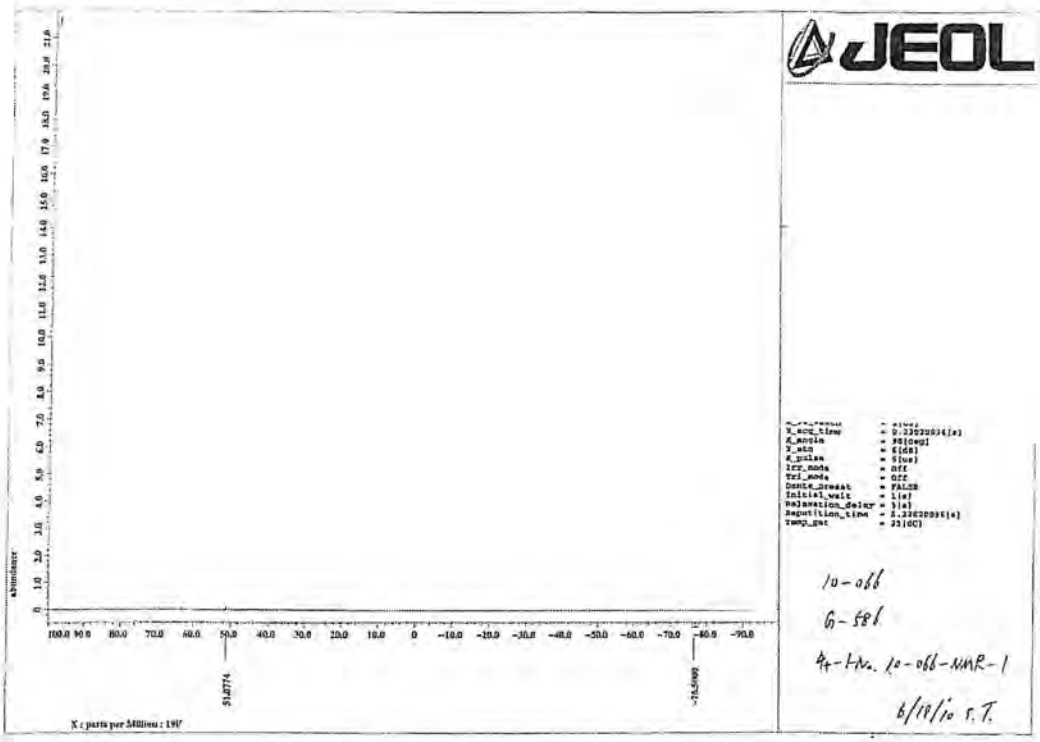

Figure 3 NMR spectrum of the test substance (after the exposure termination)

Concentration (mg/L)	Peak area	Quantitative value (mg/L)	Accuracy (%)
1.01	2006	0.956	94.7
2.02	3728	2.13	106
3.03	5151	3.11	103
4.04	6471	4.01	99.3
5.05	7830	4.94	97.8

Method: C:\MassLyn> \MethDB\ .mdb 21 Apr 2010 18:23:08
Calibration: 24 May 2010 16:38:14

定量用

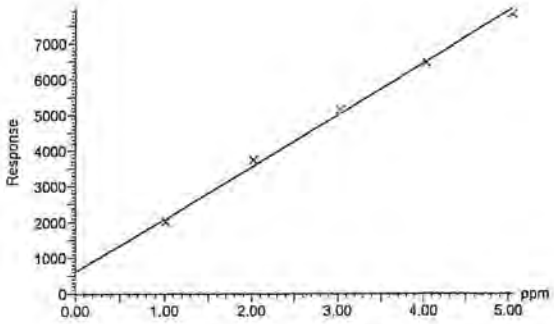

Figure 4 Typical calibration curve

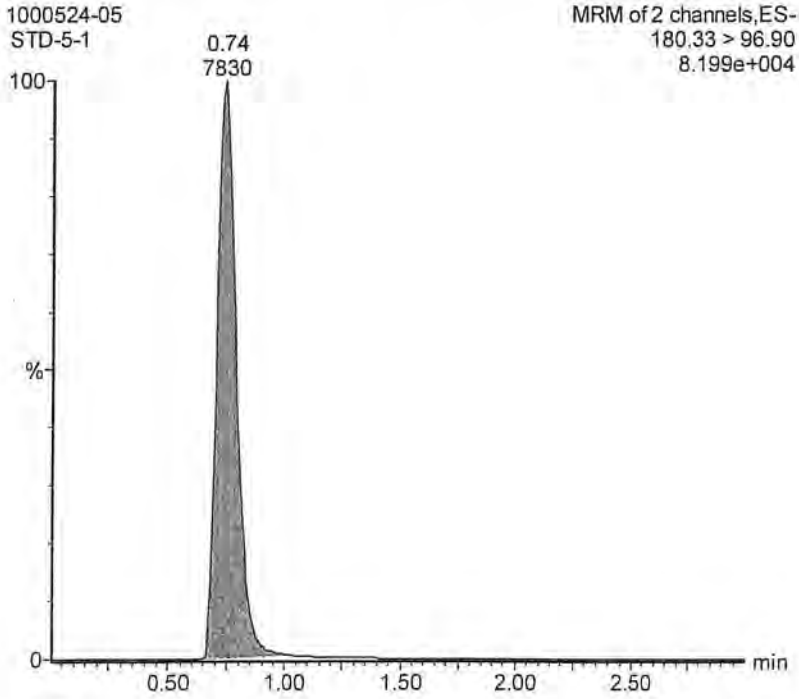

Figure 5 Typical chromatogram of a standard solution (5.05 mg/L, at the start of the exposure)

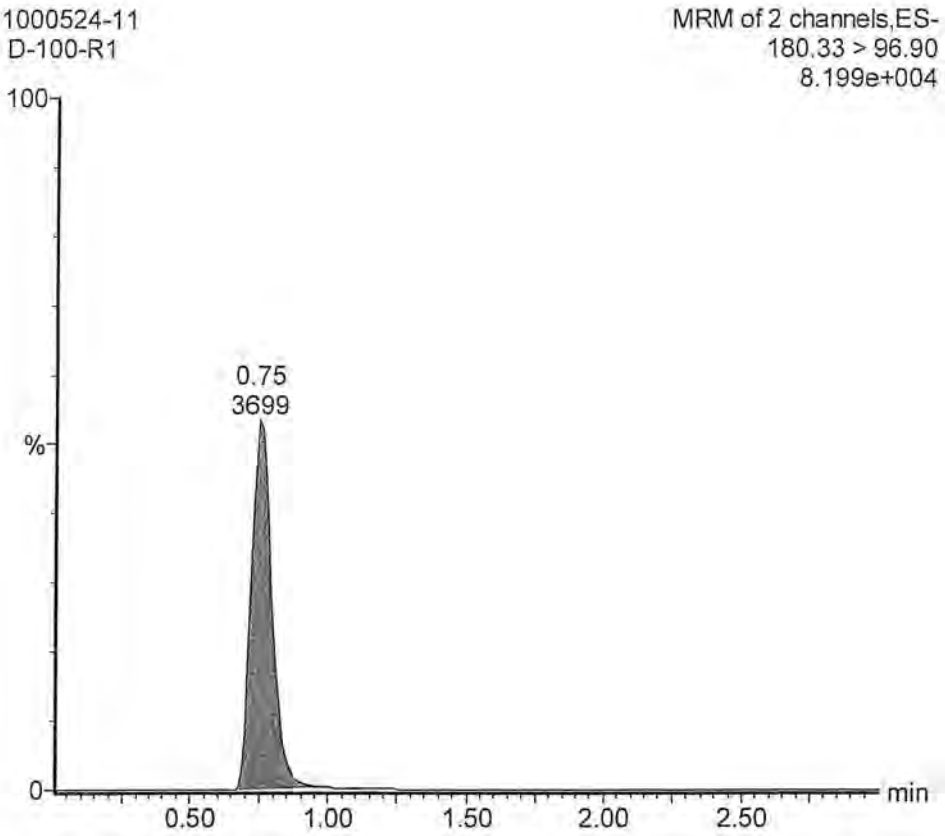

Figure 6 Typical chromatogram of a repeatability test sample (nominal concentration; 100 mg/L)

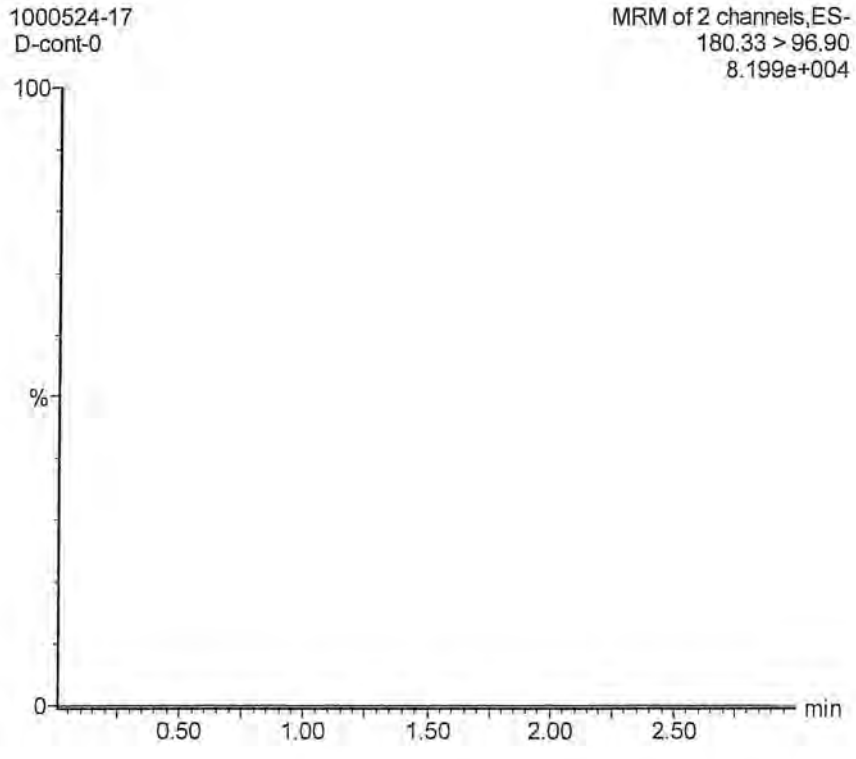

Figure 7 Chromatogram of a control solution at the start of the exposure

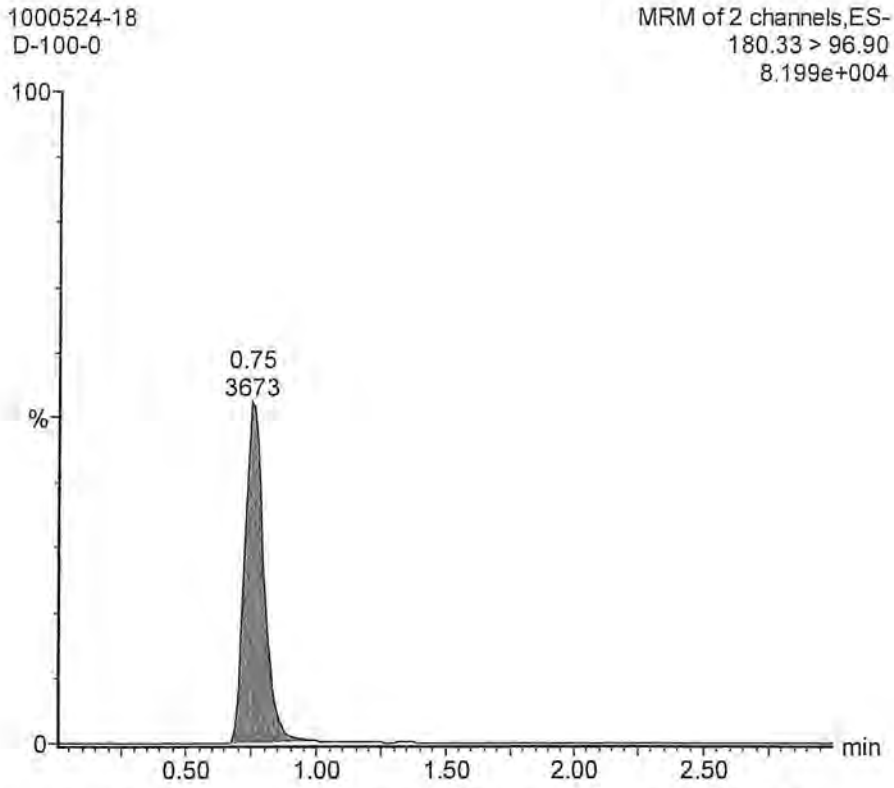

Figure 8 Chromatogram of a 100 mg/L solution at the start of the exposure

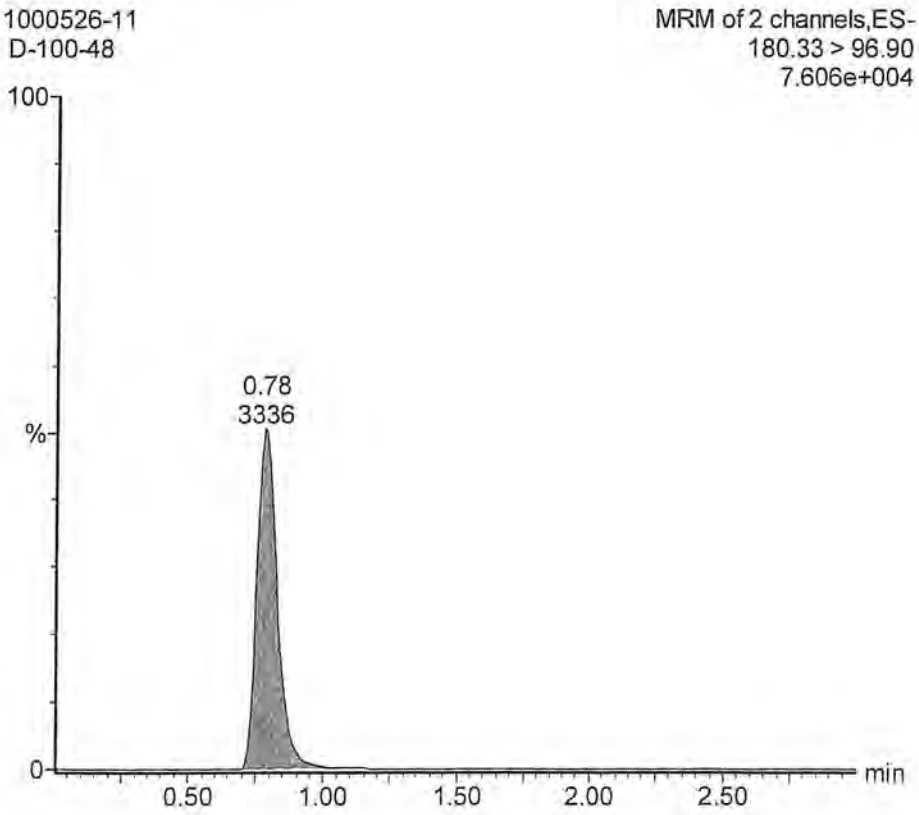

Figure 9 Chromatogram of a 100 mg/L solution at the end of the exposure

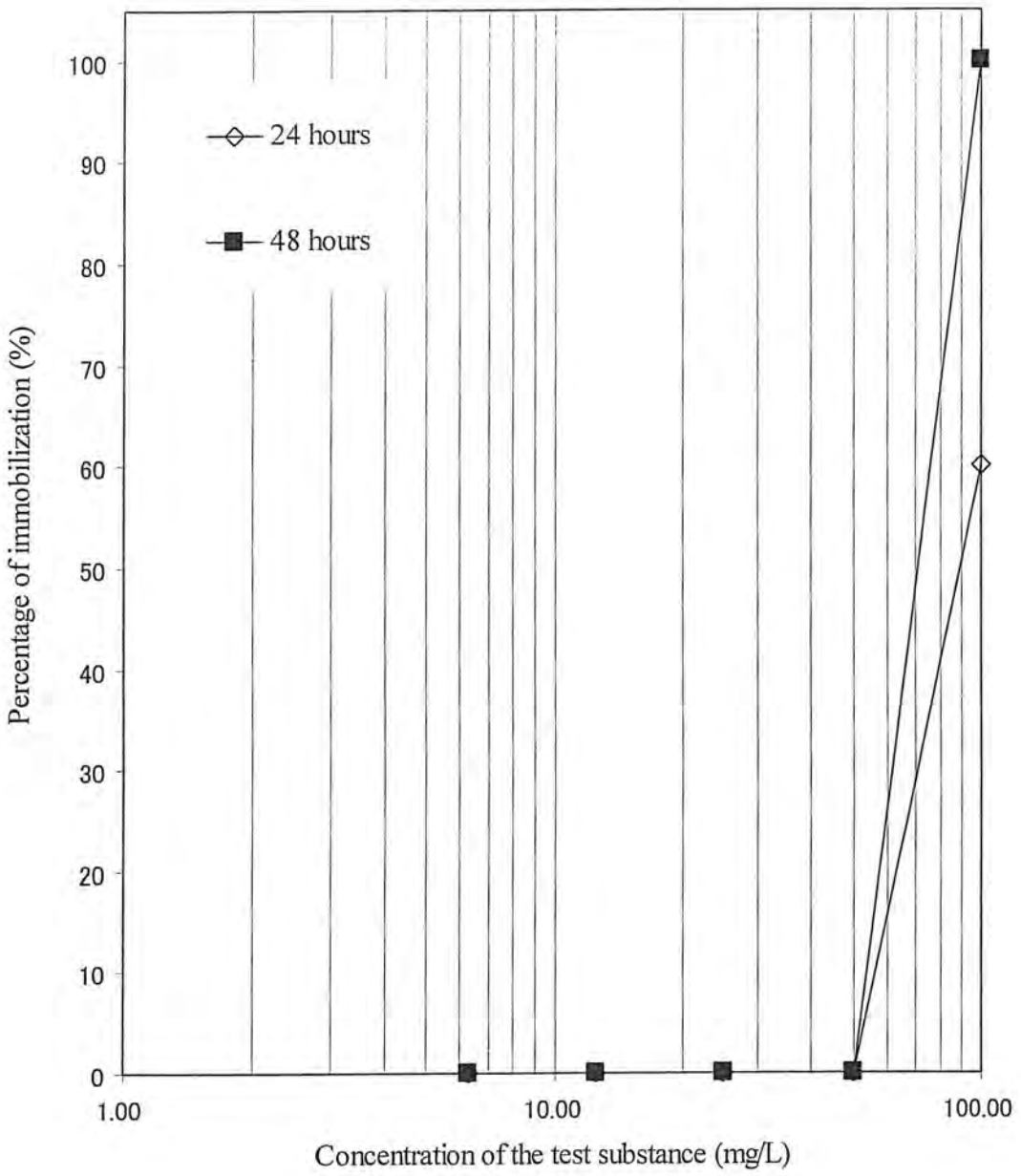

Figure 10 Concentration of the test substance and percentage of immobilization

Authenticity of Translation

I declare that the original Japanese final report (Report No. NCAS 10-064) is translated into English consistently.

Translated by : Osamu Saika June 5, 2012

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